Collections of Fungi in the United States

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PREFACE

This report gives the current status of culture collections of fungi in the United States and explores certain current, and possible future, problems facing curators of these collections as well as other microbiologists. It is intended to stimulate all who work with mold fungi in microbial fermentations, plant pathology, deterioration, and ecology to help solve problems of mutual interest.

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INTRODUCTION

Culture collections can be thought of as reservoirs of organisms and information concerning their growth and products. A. L. van Beverwijk (in Martin *1963*)[2] stated that culture collections can be of a specialized nature or of a general service nature. Of course, these distinctions overlap in many: some collections are specialized as to organism; others are specialized as to subject. Examples of these categories will be discussed. Exact addresses for the collections mentioned can be obtained from the World Directory of Collections of Microorganisms (Martin and Skerman *1972*). Not only will various collections be discussed as they exist in the United States but also some current and possible future problems will be mentioned that present themselves to those responsible for organizing and maintaining a culture collection.

CULTURE COLLECTIONS IN THE UNITED STATES

General Service Collections

The largest general service collection now in existence in the United States is The American Type Culture Collection (ATCC).[3] Although ATCC began much earlier, their culture list did not include fungi until 1925. Even then, the fungi were not maintained in the collection but were listed as "available from special collections." Certain ATCC numbers were allocated for Aspergilli and Penicillia, available from the Charles Thom Collection; other numbers were reserved for yeasts maintained by F. W. Tanner.[4]

In succeeding years, similar assignments of numbers were made for fungus cultures in individual collections.[5] By 1938, when the fourth edition of the ATCC catalog was published, the first fungi had been transferred from a private collection to the ATCC for maintenance; these were the Aspergilli from the Thom collection. In the next 11 years, the number of fungi, including yeasts, increased to over 3,000.

These fungi were useful for teaching; for the commercial production of organic acids, alcohols, sugars, antibiotics, and vitamins; for assays particularly important in the deterioration of fabrics and paint; and for production of amino acids and vitamins. Some were important to medical mycology, others to phytopathology. Sources of the strains were varied. ATCC's 1977 annual report mentioned a total of over 11,000 strains maintained by the Mycology Department.

Fungi have been incorporated into the ATCC collection in an attempt to remain as broad in interest as possible. The dominant strains are types, industrial strains, inhabitants of soil and water, and plant pathogens (including rusts). Others represent human and animal pathogens, wood deteriorators, strains for genetic studies, mycotoxin producers, strains for bioassay, edible mushrooms and yeasts, nematode-trapping fungi, virus hosts, and mycoparasites.

The dominant methods of preserving strains are by freeze-drying spores of various kinds suspended in skim milk and by freezing and storing in liquid nitrogen.

[1] Mycological curator, Agricultural Research Culture Collection (NRRL); deceased; chief, Fermentation Laboratory, Northern Regional Research Center, Science and Education Administration, Agricultural Research, U.S. Department of Agriculture, Peoria, Ill. 61604.

[2] Author's names and years (italic) refer to References p. 9.

[3] 12301 Parklawn Dr., Rockville, Md. 20852.

[4] U.S. Department of Agriculture, Washington, D.C. 20250; University of Illinois, Urbana 61801.

[5] C. Drechsler and J. Stevenson, U.S. Department of Agriculture, Beltsville, Md. 20705; C. D. Sherbakoff (Fusaria), University of Tennessee, Knoxville 37916; A. F. Blakeslee (Mucorales), Harvard University, Cambridge, Mass. 02138.

ATCC is an independent nonprofit organization. It is supported by grants, contracts, sales of cultures, and services. The current staff of the Mycology Department consists of a department head and eight full-time members. The department provides opportunities for visiting scientists for a limited tenure. Although staff members check strain identities and do taxonomic studies, a schedule for identification services must be made by arrangement with ATCC.

A 13th edition of their 1978 Catalog of Strains can be purchased from them for $10. A fee is charged for cultures of strains ordered. The procedure for depositing strains in connection with patents is outlined in the introduction to their catalog or can be obtained as a separate (ATCC Form No. 6, 5/77).

Specialized Collections

Quartermaster (QM) is the sigla for the strains that were maintained by the mycology group and Culture Collection at the U.S. Army Natick Laboratories, including those from many early contributors.[6]

The QM collection contained a variety of fungi and yeasts emphasizing tropical and subtropical isolates from such deteriorating material as cotton and wool fabrics, paper, leather, and plastic materials. The only available listing of cultures contained in the collection was published in 1950 (Reese and others *1950*) and referred to about 680 strains of fungi, including yeasts, by QM strain number. These were mostly Fungi Imperfecti and included numerous strains noted for their ability to deteriorate cellulose and wool. Notable too in this listing is a collection of *Fusarium* species identified by C. D. Sherbakoff. Many isolates have been added since that time and now number over 10,000, including many dark-spored hyphomycetes. The two main methods of preservation used were lyophilization and agar slant cultures overlaid with sterilized mineral oil. Cultures were available without charge, but the number per request was limited.

This collection was supported by the U.S. Government, but the program was phased out about 1973. E. G. Simmons, director of the QM collection, took the bulk of the collection, mostly in lyophil preparations, to the University of Massachusetts, Amherst, for maintenance. The Agricultural Research Culture Collection (NRRL) at the Northern Regional Research Center (NRRC), is currently making arrangements to incorporate the QM collection of microorganisms. Many of the QM strains have already been accessioned over the years. Other major culture collections throughout the world also contain many QM strains.

The Agricultural Research Culture Collection (NRRL) of the U.S. Department of Agriculture (USDA) is another large specialized culture collection. Its efforts are oriented toward applied research on farm commodities. The origin of the NRRL collection of fungi, including yeasts, has been well documented, and reference is made particularly to five publications: Hesseltine and others *1970*, Hesseltine and Haynes *1974*, Pridham and Hesseltine *1975*, Pridham *1977*, and Nakamura 1977. Of the approximately 56,000 strains of microorganisms in the NRRL collection, about 30,000 are in the fungus collection and more than 13,000 are in the yeast collection. The remainder are bacteria and actinomycetes. As part of their duties, three curators, each with an assistant, are responsible for preserving and maintaining the fungi and yeasts.

A large increase has resulted in Penicillia and Aspergilli over the initial Thom and K. B. Raper strains that formed the nucleus of the NRRL collection of fungi. Many of these additional strains were supplied by Raper and D. I. Fennell. Blocks of numbers are assigned to strains of *Paecilomyces*, *Scopulariopsis*, *Gliocladium*, A. F. Blakeslee's collection of Mucorales, Mucorales of India, Mucorales of Pakistan, Mucorales from oriental fermented foods, and G. F. Orr's collection of Gymnoascales. The NRRL collection also includes A. J. Mix's collection of *Taphrina*, N. C. Laffer's collection of yeasts from dairy products, J. L. Etchells' collection of yeasts from pickles and other fermented foods, J. W. Fell's collection of marine yeasts, collections of yeasts from Spain, and yeasts collected by L. J. Wickerham.[7]

[6] U.S. Army Natick Laboratories, Natick, Mass. 01760; W. H. Weston, Jr., D. H. Linder, E. S. Barghoorn, and W. L. White, Harvard University, Cambridge, Mass. 02138; K. B. Raper, University of Wisconsin, Madison 53706; G. W. Martin, University of Iowa, Iowa City 52242; G. F. Weber, University of Florida, Gainesville 32611; and W. G. Hutchinson, University of Pennsylvania, Philadelphia 19174.

[7] G. F. Orr, Dugway Proving Ground, Dugway, Utah 84022; A. J. Mix, University of Kansas, Lawrence 66044; N. C. Laffer, University of Maryland, College Park 20740; J. L. Etchells, North Carolina State University, Raleigh 27607; J. W. Fell, University of Miami, Miami, Fla. 33124; L. J. Wickerham, SEA-AR, NRRC, Peoria, Ill. 61604.

Since about 1973, members of the NRRL collection staff and associates have attempted to collect representatives of all fungi that produce mycotoxins. Currently the collection includes strains in more than 26 genera. Visiting scientists are appointed for varying periods to work on problems in the Center, and NRRC participates in cooperative student programs.

Identifications of fungi and yeasts are primarily done for scientists at NRRC; identification of isolates from others is done by arrangement with the respective curators. No catalog or list of strains is issued or available for distribution, but cultures in limited number are sent free of charge to *bona fide* research workers. Nearly all NRRC strains documented in publications and patents are available through the major general service culture collections, especially ATCC. Patent deposit strains are accepted, and the procedures are detailed in the publications cited.

USDA's Forest Service has a specialized culture collection of fungi, namely, the Reference Culture Collections of the Center for Forest Mycology Research, Forest Products Laboratory, Madison, Wis. The collection originated in 1932 when three small working collections of strains isolated by forest pathologists of USDA's Division of Forest Pathology were consolidated in Washington, D.C. The collection consists of strains of wood-rotting and wood-inhabiting species of Hymenomycetes. They are maintained as refrigerated agar slant cultures and as cultures overlaid with sterilized mineral oil.

The Center for Forest Mycology Research data sheet states "Axenic cultures of unknown Hymenomyceteous fungi isolated from wood and wood products are identified for scientists in federal and state government agencies and, by previous arrangement, for scientists in universities." Cultures are available free of charge to these same persons and to other nonprofit institutions.

The Fungal Genetics Stock Center at Humboldt State College, Arcata, Calif., is another large culture collection that specializes in the use of certain fungi but provides the accessibility of a generalized service collection. In 1960, this center began at Dartmouth College to collect, maintain, validate, and distribute mutant strains of *Neurospora*. It has continued to grow, through supporting grants from the National Science Foundation, "to provide the services of collecting, maintaining, improving, and distributing mutant stock strains of *Neurospora crassa, Aspergillus nidulans, Sordaria fimicola,* and related organisms" of a nonpathogenic nature. Approximately 2,700 strains are preserved primarily on sterile anhydrous silica gel and by lyophilization, and they are maintained by a curator and part-time supporting staff. Facilities have been made available for visiting scientists to work for a limited time. No identification service is available, but stock cultures are verified by the staff. Culture lists are published annually, and a limited number of cultures can be ordered from the collection without charge. An exchange or contribution is welcomed, however.

The *Fusarium* Center, Department of Plant Pathology, Pennsylvania State University, University Park, is another specialized center of activity. The collection, started in 1966 by P. E. Nelson and T. A. Toussoun, and the Center were officially recognized by the University and established in 1970. More than 5,000 isolates of *Fusarium* species are contained in the collection and preserved primarily as lyophilized preparations or as soil cultures. Strains include plant and animal pathogens, saprobes, and mycotoxin producers; a few produce plant growth-promoting substances. Although no list of cultures has been published, identifications are provided through arrangement with the curator, and many isolates have been made available to other collections.

University and College Collections

Culture collections in numerous universities and colleges throughout the United States vary in size and permanency. They range from the large general collection maintained for many years in the Department of Bacteriology at the University of Wisconsin, Madison, to many small temporary collections briefly kept either for teaching purposes or for immediate research use.

The culture collection at the University of Wisconsin (WB sigla) numbers more than 4,000 strains of general, agricultural, or industrial interest. For many years members of the department carried out a program of isolating fungi from forest soils, and the collection is particularly known for its representatives of the Aspergilli and Acrasieae. This collection is funded by the University. No catalog is published, but an exchange of cultures free of charge is made with other collections. Some cultures are distributed through ATCC, and a few cultures occasionally may be distributed to certain collectors on request.

A small but quite specialized collection of fungi

and yeasts is maintained at the College of Veterinary Medicine, University of Illinois, Urbana. About 60 fungi and 15 yeasts are kept there primarily for research and teaching purposes for those interested in veterinary microbiology. These are just a few strains included in a much larger collection of microorganisms of the same general interest.

Cultures from the smaller collections at universities and colleges can frequently be obtained from the larger general service culture collections or from the individual investigator. We shall mention certain individual investigator's collections later.

Collections of Medical Interest

Four culture collections might be grouped as having primarily health-related interests. The Mycology Section Culture Collection of the Center for Disease Control is a part of the Department of Health, Education, and Welfare, U.S. Public Health Service, Atlanta, Ga. It contains over 2,400 strains of fungi of medical interest maintained by the curator, part-time professional, and full-time technician. The cultures are primarily for research and teaching, but certain cultures can be obtained free of charge by research workers. No catalog of cultures is issued, and routine requests are referred to ATCC.

A collection of approximately 600 fungi has been maintained at the Mycology Research Laboratory of the Veterans Administration Hospital, San Fernando, Calif. A specialist collection of *Coccidioides* was accumulated there. A curator, a full-time professional, and a full-time technician maintain the collections, which are U.S. Government supported. No catalog is issued, but cultures are distributed free of charge to other culture collections.

About 250 fungi and about 75 yeasts of medical interest are maintained in a third U.S. Government supported collection, namely, the Collection of the Medical Mycology Section, National Institutes of Health, Bethesda, Md. These strains were gathered primarily for research in medical microbiology.

The Mycological Culture Collection, Division of Laboratories and Research, New York State Department of Health, Albany, is another collection oriented in research and teaching of medical microbiology. This state government-sponsored collection is the responsibility of a full-time curator and consists of approximately 800 fungi, including yeasts. Cultures are distributed to other culture collections, but no listing of strains is issued.

Industrial Collections

Certain industrial concerns support and maintain collections of fungi for use in their research, screening programs, and production of microbial products. Catalogs or lists of cultures are not published, but some investigators will exchange cultures. Cultures trickle out to the other culture collections and the general public through depositing strains for patent purposes and issuance of patents mentioning deposited strains. At any one time a concern may screen many thousands of microorganisms for a particular activity, but only a few may be kept for any length of time. In other instances rather large collections of strains are maintained. Little or no provisions are available for visiting scientists to study, and identification services are restricted to their own interests.

The World Directory of Collections of Cultures of Microorganisms (Martin and Skerman *1972*) lists 10 industrial concerns in the United States that maintain from about 50 to more than 5,500 strains of fungi in their microbial culture collections. These numbers, of course, are now 6 years old and presumably outdated. Two examples will be mentioned.

The collection of fungi at the Lilly Research Laboratories,[8] Indianapolis, Ind., began in 1940. This collection, now numbering approximately 8,000 strains, consists mostly of fungus strains known to produce antibiotics; however, a few plant pathogenic fungi are also maintained for testing. Strains surviving the process are maintained by lyophilization; strains not surviving lyophilization are placed in the vapor phase of liquid nitrogen using a glycerol-lactose suspending agent. As is the case with most larger industrial collections, the Lilly collection of fungi is maintained by a curator and a technician with the help of professional staff members.

The Upjohn Co. of Kalamazoo, Mich., has a collection dating from 1954 of about 2,200 strains —many of pathogenic, antibiotic, steroid, or assay interest. The preferred ways of preservation are as soil stocks and as agar plugs stored in the gas phase of liquid nitrogen. The collection is maintained by a curator and technical assistant.

[8] The mention of firm names or trade products does not imply that they are endorsed or recommended by the U.S. Department of Agriculture over other firms or similar products not mentioned.

Personal Collections

The following listings are collections of individual mycologists. They are not intended to be all-inclusive but include predominantly selective groups of strains in important specialized personal collections located mainly in universities and colleges.

About 1,500 isolates of fungi are maintained as agar slant cultures by R. K. Benjamin at Rancho Santa Ana Botanic Garden, Claremont, Calif. This collection, started in 1953, is strong in Mucorales and allied fungi as well as numerous Gymnoascaceae. Especially well represented are members of the Thamnidiaceae and merosporangiferous Mucorales.

At the Plant Pathology Department, University of Illinois, Urbana, J. W. Gerdemann maintains a collection of Endogonaceae species. These have not yet been obtained in pure culture and, therefore, are maintained by growth in association with the roots of angiosperms growing in pots. In the Botany Department, C. A. Shearer has a research collection of about 200 strains of fresh-water and marine Hyphomycetes and Ascomycetes.

About 900 strains, many of fleshy fungi, are maintained in L. R. Kneebone's Mushroom Laboratory at Pennsylvania State University, University Park. This collection was started in 1930 by J. W. Sinden. Stock cultures from this collection were progenitors of well over half the original strains used in commercial mushroom growing in the United States and Canada. Other cultures in this collection are associated with edible mushroom production such as mushroom pathogens, competitors, and compost microorganisms. They are maintained by regular transfer in culture tubes.

At the Department of Botany, University of Kansas, Lawrence, R. W. Lichtwardt maintains a collection of about 60 isolates of Trichomycetes. They are stored in active; but refrigerated, cultures and in liquid nitrogen.

At the Department of Plant Pathology and Plant Genetics, University of Georgia, College of Agriculture, Athens, E. S. Luttrell has accumulated some 200 isolates of *Helminthosporium* and related genera.

Approximately 200 strains of fungi, most plant pathogens, have been collected by J. L. Peterson, Department of Plant Pathology, Rutgers University, New Brunswick, N.J.

At the New York Botanical Garden, the Bronx, C. T. Rogerson maintains a collection of some 2,000 isolates of predominantly hypocrealean and fungicolous fungi as refrigerated agar slant cultures. There, too, are some 36 ant fungi obtained from Neil Weber.

These examples represent culture collections, large or small, generalized or specific, from the viewpoint of use and contents.

SOME MAJOR PROBLEMS

Certain problem areas that confront curators of fungus culture collections can be placed in three broad categories: acquisition, maintenance, and distribution. One problem of a general nature is evident from the foregoing discussion of specialists' collections. When possible, cultures from the nomenclatural types of species are deposited in one or more of the major culture collections. What happens, however, to a worker's overall collection when he goes on to another project, loses his funding, or retires and no longer has help to maintain many cultures? Potentially valuable cultures that have been carefully examined and identified may be lost under these circumstances. Such cultures might be screened by a panel to determine which should be preserved in a permanent culture collection.

The following areas also need some thoughtful consideration.

Plant Pathogenic Fungi

A major culture collection of plant pathogens may be needed in light of the increased importance, the use in assay procedures, and the possible production by some of desirable or undesirable by-products. Admittedly, many of these fungi are quite difficult to maintain; others currently are not growing in pure culture. The ATCC has numerous plant pathogens, including a large collection of rusts; but so far, no collection is collecting, studying, maintaining, and making available cultures of all possible plant pathogens. Perhaps phytopathologists would be interested in arranging an accumulation of these strains and supporting such an endeavor.

Mycorrhizal Fungi

A long and continued study of mycorrhizal fungi exists in relation to establishing and growing conifers in forest regeneration. Research also is continuing to improve the quality of nursery hardwood seedlings and their field performance from associations with mycorrhizal fungi. Despite the increasing interest in this group of fungi and their undoubted importance, no concerted effort has been made to devise a culture collection reservoir of all mycorrhizal fungi.

Deposit of Type Strains

A less obvious problem concerns living type strains. Both taxonomists and curators are now or will be confronted with facets of the designation and deposition of living type strains, primarily with the types of species and varieties.

Article 7 of the International Code of Botanical Nomenclature (*1972*) states: "A *holotype* is the one specimen or other element used by the author or designated by him as the nomenclatural type."

Recommendation 7A states: "It is strongly recommended that the material on which the name of a taxon is based, especially the holotype, be deposited in a permanent, responsible institution and that it be scrupulously conserved. When living material is designated as a nomenclatural type (for Bacteria only; see Art. 9, paragraph 3), appropriate parts of it should be immediately preserved."

Article 9, paragraph 3 states: "Type specimens of names of taxa, the Bacteria excepted, must be preserved permanently and cannot be living plants or cultures."

Consider, for the moment, what the bacteriologists have done. The International Code of Nomenclature of Bacteria (*1975*) quite clearly states:

Rule 18a: "Whenever possible the type of a species or subspecies is a designated strain.

"A type strain is made up of living cultures of an organism which are descended from a strain designated (except as in Rule 18c) as the nomenclatural type. The strain should have been maintained in pure culture and should agree closely in its characters with those in the original description (see Chapter 4, C). The type strain may be designated in various ways (see Rules 18b, c, d, e, and f).

"For a species which has not so far been maintained in laboratory culture or for which a type strain does not exist, a description, preserved specimen, or an illustration (see also Rule 18h) may serve as the type."

Rule 18e *Designation as neotype:* "If a strain on which the original description was based cannot be found, a neotype strain may be proposed.

"A neotype strain must be proposed (proposed neotype) in the IJSB [International Journal of Systematic Bacteriology], together with citation of the author(s) of the name, a description or reference to an effectively published description, and a record of the permanently established culture collection(s) where the strain is deposited (see also Note 1 to Rule 24a).

"The author should show that a careful search for the strains used in the original description has been made and that none of them can be found. The author should also demonstrate that the proposed neotype agrees closely with the description given by the original author.

"The neotype becomes established (established neotype) two years after the date of its publication in the IJSB, provided that there are no objections, which must be referred within the first year of the publication of the neotype to the Judicial Commission for consideration."

Rule 18h: "If a description or illustration constitutes, or a dead preserved specimen has been designated as the type of a species (Rule 18a, para 3) and later a strain of this species is cultivated, then the type strain may be designated by the person who isolated the strain or by a subsequent author. This type strain shall then replace the description, illustration, or preserved specimen as the nomenclatural type."

Arguments can be proposed for and against designating and depositing a living type strain as the nomenclatural type for a species of fungi. Currently, characteristics of some fungi are known only from culture. Many examples could be cited from fungi originating from soil debris and animal sources and from yeasts.

Dried type herbarium specimens have never or no longer exist for many Phycomycetes, Zygomycetes, yeasts, and hyphomycetes. In these groups our species concepts often reflect responses to the growth of a strain on specific reproducible media. Pigmentation, branching, exudate, and growth rate frequently are used.

Possibilities presented for type material are that it be (1) a dried preserved specimen, (2) a well-documented illustration, (3) a living strain, and (4) any combination of the three. Each has its advantages and disadvantages. The first two materials are, within reason, nonchanging and static.

The third possibility, a living strain, is in a dynamic state and preferably maintained under conditions such that the strain is always capable of expressing its original genotype.

Certain dried herbarium specimens of filamentous fungi are quite useful and contain much information. An example is *Syzygites* growing on the fruiting body of a Basidiomycete. In other instances, including the majority of the Mucorales, dried herbarium specimens either are low in taxonomic value or no longer exist. The branching pattern of certain Mucorales may become incomprehensible in the tangled mass of the dried specimen. Physiological characteristics cannot be further determined unless the sporangiospores are still viable and a culture is established.

For others, such as the Penicillia and Aspergilli, the colony color eventually fades in most specimens after drying. Colony color and mass conidial color are helpful characteristics in distinguishing species within these two genera. A specimen of some of the smaller Phycomycetes may lose much of its usefulness when dried because many thalli break away from the substrate and are lost.

On the other hand, a dried herbarium specimen often contains a wealth of morphological information. With the advent of the scanning electron microscope, more information than ever before can be obtained from the examination of such material. As more sophisticated instruments are developed and become available, more helpful information will be obtained from dried specimens that currently are nearly useless. Minute portions of a specimen can be analyzed through micro methods either microchemically or physically with chromatography and spectrometry.

Undoubtedly, these techniques will be used to a greater extent not only on living cultures but also on dried specimens. A living strain will give more information by these techniques than will a dried specimen because a living strain can be analyzed at nearly any stage of morphological development or growth and with a choice amount of material. With a living strain, the probability of discovering new features is greater than those apparent or available at the time the original collection or isolate was studied.

Assuming that a living type strain becomes acceptable as the nomenclatural type and that a dried specimen is prepared that shows the morphological features typifying the taxon, which of the two elements typifies the taxon if the living type changes? If the living type should lose certain morphological features, it does not present as great a problem as would be encountered should it exhibit new morphological features not found by examining the dried specimen.

An example might be the formation of the perfect stage. This new feature should be preserved also as a dried specimen because it may occur sporadically or be difficult to reproduce. The status of the newly dried specimen then must be determined. A living type strain must be carefully defined. Perhaps a goal to attain would be to preserve and maintain the dynamic potential of the type strain in such a way that it will always express optimum features needed to typify the taxon.

If living type strains are designated as nomenclatural types, perhaps neotype strains should be designated where type strains do not exist. Further problems would arise when a nomenclatural type exists as a dried specimen and a neotype strain is not acceptable according to the current Botanical Code.

What can be done with heterothallic, haploid species? If a species of a heterothallic Zygomycete is to be described and a dried specimen deposited to conform to the present Botanical Code, what is dried for the holotype? Is it correct to designate and deposit a dried specimen of the (+) or (−) strain or a Petri dish culture of the contrasted pair that contains zygospores to represent the nomenclatural type? Perhaps the whole organism, when available, should be deposited and not just a part. But then would this be inconsistent? Would this designate a pair of strains in one instance and only a single strain in another instance when the zygosporic stage is as yet unknown? Perhaps we should consider a statement similar to Wickerham's (*1965*) attempt to clarify the situation by proposing for haploid, heterothallic yeasts: "the type specimen of a heterothallic haploid species of yeast shall consist of two strains of opposite sex. One of them may be the type of the corresponding imperfect state."

In another aspect of the same problem a single strain of a heterothallic Zygomycete may have been preserved as a dried specimen, but, when one finds the opposite mating strain, little chance remains to observe the perfect state if a living strain from the original type has not also been preserved.

A final area in this overall theme is a matter that concerns both mycologists and curators of culture collections, namely, one of obligation. The investigating mycologist should designate and deposit

holotypes; however, if living type strains are involved, what actually is the holotype? It could be an agar slant culture or a set of lyophil ampules of a culture made as a single batch. If it is an agar slant culture and members of the culture collection prepare ampules by lyophilization, these preparations represent new elements and should be carefully checked by the depositor and their status determined. The obligations of curators and culture collections are many, including (1) receiving and preserving under optimal conditions the total genotype received, (2) making authentic preparations from the material received, and (3) renewing the strain when all original ampules are exhausted, when change has occurred, or when the culture is no longer viable.

Should curators also be obliged to preserve dried specimens when new features, such as formation of the perfect state, arise? What status, then, does the newly dried specimen have? The International Mycological Association (IMA) also must share obligations in certain matters, for example, ruling on the acceptance of neotype strains. Thus, all three groups have obligations, namely, the investigators, the curators and culture collections, and the IMA.

Despite these seemingly insurmountable problems, many can be resolved. The Botanical Code does not prohibit the designation of a type strain by number. Therefore, in addition to the deposition of a dried herbarium specimen as the nomenclatural type, a living type strain should be designated whenever possible, and every attempt should be made to maintain cultures in as original a form as possible.

Patent Deposit Strains

This final topic concerns recent developments in relation to the deposition of patent strains. As stated earlier, many facets, including NRRC policies, have been detailed in previous publications (Hesseltine and others *1970;* Pridham and Hesseltine *1975*). A document was prepared by the International Bureau of the World Intellectual Property Organization (WIPO) entitled "Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure" (*1977*). This treaty was signed by Bulgaria, Denmark, Finland, France, Federal Republic of Germany, Hungary, Italy, Netherlands, Norway, Spain, Switzerland, United Kingdom, and the United States. It consists of 20 articles followed by 15 rules having the following headings:

Rule 1. Abbreviated Expressions and Interpretation of the Word "Signature"
Rule 2. International Depositary Authorities
Rule 3. Acquisition of the Status of International Depositary Authority
Rule 4. Termination or Limitation of the Status of International Depositary Authority
Rule 5. Defaults by the International Depositary Authority
Rule 6 Making the Original Deposit or New Deposit
Rule 7. Receipt
Rule 8. Later Indication or Amendment of the Scientific Description or Proposed Taxonomic Designation or Both
Rule 9. Storage of Microorganisms
Rule 10. Viability Test and Statement
Rule 11. Furnishing of Samples
Rule 12. Fees
Rule 13. Publication by the International Bureau
Rule 14. Expenses of Delegations
Rule 15. Absence of Quorum in the Assembly

This treaty attempts to reduce or eliminate more than one deposit of the same strain of microorganism when protection is sought in more than one country for an invention involving the use of a microorganism. A provision of the document is that the contracting States recognize, for purposes of patent procedure, the fact of deposit and date of deposit as indicated by an international depositary authority.

An international depositary authority is defined as a depositary institution "which has acquired the status of international depositary authority" by appropriate written communication to the director general of WIPO and the publication of that communication. This matter might seem at first glance to be one of concern to only a few culture collections. It does, however, involve the care and availability of a growing number of strains already in the thousands.

This treaty might be considered from three or possibly four standpoints, namely, obligations of the depositor, those of the culture collection, those of the requestor, and the role, if any, of the IMA. For the present, the authors wish to consider some obligations of the culture collection, which acts as an international depositary authority.

The depositary institution provides for (1) the

receipt, (2) the acceptance, (3) the storage, and (4) the furnishing of samples (for example, living cultures) of microorganisms deposited with it. The culture collection must have the capability of storing those microorganisms deposited with it and a continuing existence because Rule 9.1 states that a deposited microorganism shall be stored in a viable and uncontaminated condition for at least 30 years.

If a culture collection cannot furnish samples of the deposited microorganism (for example, if the microorganism should be no longer viable), provision is made in Article 4 of the treaty for a "new deposit" of the microorganism originally deposited. According to Rule 10, the culture collection must test the viability of each microorganism at the time of deposit and at reasonable intervals. A viability test might also be required at other times upon request of the depositor.

Of perhaps wider interest is the availability of deposited microorganisms especially those new to the scientific literature. Provision is made by Rule 11 to furnish samples upon request to an industrial property office of a contracting State, to the depositor, to a party authorized by the depositor, or to parties legally entitled to receive a sample. Any party not entitled to receive a sample is, likewise, not entitled to receive information about a deposited microorganism. Effectively, information about microorganisms deposited for patent purposes becomes available with the issuance of the patent. Because the treaty and regulations apply to a large and growing number of strains, they deserve careful attention.

REFERENCES

Budapest treaty on the international recognition of the deposit of microorganisms for the purposes of patent procedure. 1977. Documents issued after the diplomatic conference held in Budapest from April 14-28, 1977. World Intellectual Property Organization, Geneva.

Hesseltine, C. W., and W. C. Haynes. 1974. Sources and management of micro-organisms for the development of a fermentation industry. U.S. Department of Agriculture, Agricultural Handbook No. 440, 38 pp.

——— W. C. Haynes, L. J. Wickerham, and J. J. Ellis. 1970. History, policy and significance of the ARS Culture Collection. *In* Culture collections of microorganisms. H. Iizuka and T. Hasegawa, editors. University of Tokyo Press, pp. 21-38.

International code of botanical nomenclature. 1972. Published for the international bureau for plant taxonomy and nomenclature by A. Oosthoek's Uitgeversmaatschappij N. V. Utrecht, The Netherlands. 426 pp.

International code of nomenclature of bacteria. 1975. Published for the international association of microbiological societies by the American Society for Microbiology, Washington, D.C. 180 pp.

Martin, S. M., editor. 1963. Culture collections: perspectives and problems. University of Toronto Press. 221 pp.

——— and V. B. D. Skerman, editors. 1972. World directory of collections of cultures of microorganisms. Wiley and Sons, Inc., N.Y. 560 pp.

Nakamura, L. K. 1977. Patent deposition of microorganisms: Agricultural Research Service. Developments in Industrial Microbiology 19:161-167.

Pridham, T. G. 1977. Fungi and bacteria, ARS Culture Collection. *In* Systematic collections of the Agricultural Research Service. W. H. Cross and others, editors. U.S. Department of Agriculture, Miscellaneous Publication No. 1343, pp. 7-13.

——— and C. W. Hesseltine. 1975. Culture collections and patent depositions. Advances in Applied Microbiology 19:1-23.

Reese, E. T., H. S. Levinson, and M. H. Downing. 1950. Quartermaster culture collection. Farlowia 4(1):45-86.

Wickerham, L. J. 1965. Opposite sexes as type specimen for heterothallic haploid yeasts. Taxon 14(6):187-188.

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